

CLAIMS

What is claimed:

1. A unicellular organism for producing a diterpene, comprising:

an exogenous nucleic acid sequence encoding a geranylgeranyl pyrophosphate synthase under the control of a promoter operable in said organism; and

an exogenous nucleic acid sequence encoding a diterpene synthase under the control of a promoter operable in said organism.
2. The unicellular organism of claim 1, wherein said nucleic acid sequence encoding said geranylgeranyl pyrophosphate synthase is SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 or SEQ ID NO:21.
3. The unicellular organism of claim 1, wherein said nucleic acid sequence encoding said diterpene synthase is SEQ ID NO:361; SEQ ID NO:362; SEQ ID NO:412; SEQ ID NO:363; SEQ ID NO:364; SEQ ID NO:365; SEQ ID NO:366; SEQ ID NO:367; SEQ ID NO:368; SEQ ID NO:369; SEQ ID NO:370; SEQ ID NO:371; SEQ ID NO:372; SEQ ID NO:373; SEQ ID NO:374; SEQ ID NO:375; SEQ ID NO:376; SEQ ID NO:377; SEQ ID NO:378; SEQ ID NO:379; SEQ ID NO:380; SEQ ID NO:381; SEQ ID NO:382 or SEQ ID NO:397.
4. The unicellular organism of claim 1, wherein said promoter of said nucleic acid sequence encoding said geranylgeranyl pyrophosphate synthase is an inducible promoter or a constitutive promoter.
5. The unicellular organism of claim 4, wherein said inducible promoter is selected from the group consisting of GAL1, CUP1 and MET3.
6. The unicellular organism of claim 4, wherein said constitutive promoter is selected from the group consisting of ADH and PGK.

7. The unicellular organism of claim 1, wherein said promoter of said nucleic acid sequence encoding said diterpene synthase is an inducible promoter or a constitutive promoter.
8. The unicellular organism of claim 7, wherein said inducible promoter is selected from the group consisting of GAL1, CUP1 and MET3.
9. The unicellular organism of claim 7, wherein said constitutive promoter is selected from the group consisting of ADH and PGK.
10. The unicellular organism of claim 1, wherein said unicellular organism further comprises an exogenous nucleic acid sequence encoding a soluble form of an HMG-CoA reductase under control of a promoter operable in said organism.
11. The unicellular organism of claim 10, wherein said promoter is an inducible promoter or a constitutive promoter.
12. The unicellular organism of claim 11, wherein said inducible promoter is selected from the group consisting of GAL1, CUP1 and MET3.
13. The unicellular organism of claim 11, wherein said constitutive promoter is selected from the group consisting of ADH and PGK.
14. The unicellular organism of claim 10, wherein said unicellular organism further comprises an exogenous nucleic acid sequence that confers to said organism an increase in sterol metabolic flux as compared to native sterol metabolic flux levels.
15. The unicellular organism of claim 1, wherein said nucleic acid sequence encoding said geranylgeranyl pyrophosphate synthase is present on a chromosome of said unicellular organism.
16. The unicellular organism of claim 1, wherein said unicellular organism is a yeast.
17. The unicellular organism of claim 10, wherein said unicellular organism is a yeast.
18. The unicellular organism of claim 14, wherein said unicellular organism is a yeast.
19. A unicellular organism for producing a diterpene precursor, comprising:

an exogenous nucleic acid sequence encoding a geranylgeranyl pyrophosphate synthase under the control of an inducible promoter operable in said organism;

an exogenous nucleic acid sequence encoding a soluble form of HMG-CoA reductase under control of an inducible promoter operable in said organism and

an exogenous nucleic acid sequence that confers to said cell an increase in sterol metabolic flux as compared to native sterol metabolic flux levels.

20. The unicellular organism of claim 19, wherein said nucleic acid sequence encoding said geranylgeranyl pyrophosphate synthase is SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 or SEQ ID NO:21.

21. The unicellular organism of claim 19, wherein said organism is a yeast.

22. A unicellular organism for producing a diterpene precursor, comprising:

an exogenous nucleic acid sequence encoding a geranylgeranyl pyrophosphate synthase under the control of an inducible promoter operable in said organism;

an exogenous nucleic acid sequence encoding a soluble form of HMG-CoA reductase under control of an inducible promoter operable in said organism and

a *upc2-1* nucleic acid sequence.

23. The unicellular organism of claim 22, wherein said nucleic acid sequence encoding said geranylgeranyl pyrophosphate synthase is SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 or SEQ ID NO:21.

24. The unicellular organism of claim 22, wherein said organism is a yeast.

25. A unicellular organism for producing a diterpene or diterpene precursor, comprising:

an exogenous nucleic acid sequence encoding a geranylgeranyl pyrophosphate synthase under the control of an inducible promoter operable in said organism;

an exogenous nucleic acid sequence encoding a diterpene synthase under the control of an inducible promoter operable in said organism;

an exogenous nucleic acid sequence encoding a soluble form of HMG-CoA reductase under control of an inducible promoter operable in said organism; and

a *upc2-1* nucleic acid sequence.

26. The unicellular organism of claim 25, wherein said nucleic acid sequence encoding said geranylgeranyl pyrophosphate synthase is SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20 or SEQ ID NO:21.

27. The unicellular organism of claim 25, wherein said nucleic acid sequence encoding said diterpene synthase is SEQ ID NO:361; SEQ ID NO:362; SEQ ID NO:412; SEQ ID NO:363; SEQ ID NO:364; SEQ ID NO:365; SEQ ID NO:366; SEQ ID NO:367; SEQ ID NO:368; SEQ ID NO:369; SEQ ID NO:370; SEQ ID NO:371; SEQ ID NO:372; SEQ ID NO:373; SEQ ID NO:374; SEQ ID NO:375; SEQ ID NO:376; SEQ ID NO:377; SEQ ID NO:378; SEQ ID NO:379; SEQ ID NO:380; SEQ ID NO:381; SEQ ID NO:382 or SEQ ID NO:397.

28. The unicellular organism of claim 25, wherein said organism is a yeast.

29. A unicellular organism for producing a diterpene or diterpene precursor, comprising:

an exogenous polynucleotide sequence encoding a polypeptide having an amino acid sequence of a geranylgeranyl pyrophosphate synthase under the control of a promoter operable in said organism;

an exogenous polynucleotide sequence encoding a polypeptide having an amino acid sequence of a diterpene synthase under the control of a promoter operable in said organism;

an exogenous polynucleotide sequence encoding a polypeptide having an amino acid sequence of a soluble form of HMG-CoA reductase under control of a promoter operable in said organism; and

an exogenous polynucleotide sequence encoding a polypeptide having an amino acid sequence of gene that confers to said organism an increase in sterol metabolic flux as compared to native sterol metabolic flux levels.

30. The unicellular organism of claim 29, wherein said amino acid sequence of said geranylgeranyl pyrophosphate synthase is SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84 or SEQ ID NO:85.

31. The unicellular organism of claim 29, wherein said amino acid sequence of said diterpene synthase is SEQ ID NO:383; SEQ ID NO:384; SEQ ID NO:385; SEQ ID NO:386; SEQ ID NO:387; SEQ ID NO:388; SEQ ID NO:389; SEQ ID NO:390; SEQ ID NO:391; SEQ ID NO:392; SEQ ID NO:393; SEQ ID NO:394; SEQ ID NO:395; SEQ ID NO:396 or SEQ ID NO:398.

32. The unicellular organism of claim 29, wherein said organism is a yeast.

33. A method of producing a diterpene, comprising the steps of:

growing a culture of cells, wherein at least one cell in said culture is the unicellular organism of claim 1, under conditions wherein said diterpene is produced; and

removing said diterpene from said culture.

34. The method of claim 33, wherein said growing step occurs in the presence of a polyaromatic resin.

35. The method of claim 34, wherein said polyaromatic resin is in a weight-to-volume ratio of at least about 5%.

36. The method of claim 33, wherein said removal comprises filtration or extraction.

37. The method of claim 33, wherein said organism is a yeast.

38. The method of claim 37, wherein said promoter of the nucleic acid sequence encoding said GGPP synthase and said promoter of the nucleic acid sequence encoding said diterpene synthase are both inducible GAL1 promoters, and wherein the growing step occurs in the presence of at least about 2% galactose.

39. A method of producing a diterpene, comprising the steps of:

growing a culture of cells, wherein at least one cell in said culture is the unicellular organism of claim 10, under conditions wherein said diterpene is produced; and

removing said diterpene from said culture.

40. The method of claim 39, wherein said growing step occurs in the presence of a polyaromatic resin.

41. The method of claim 40, wherein said polyaromatic resin is in a weight-to-volume ratio of about 5%.

42. The method of claim 39, wherein said removal comprises filtration or extraction.

43. The method of claim 39, wherein said cell is a yeast.

44. The method of claim 43, wherein said promoter of the nucleic acid sequence encoding said GGPP synthase, the promoter of the nucleic acid sequence encoding said diterpene synthase and the promoter of the nucleic acid sequence encoding said soluble form of said HMG-CoA reductase are inducible GAL1 promoters, and wherein the growing step occurs in the presence of at least about 2% galactose.

45. A method of producing a diterpene, comprising the steps of:

growing a culture of cells, wherein at least one cell in said culture is the unicellular organism of claim 14, under conditions wherein said diterpene is produced; and

removing said diterpene from said culture.

46. The method of claim 45, wherein said growing step occurs in the presence of a polyaromatic resin.

47. The method of claim 46, wherein said polyaromatic resin is in a weight-to-volume ratio of about 5%.

48. The method of claim 45, wherein said cell is a yeast.

49. The method of claim 48, wherein said promoter of the nucleic acid sequence encoding said GGPP synthase, the promoter of the nucleic acid sequence encoding said diterpene synthase and the promoter of the nucleic acid sequence encoding said soluble form of said HMG-CoA reductase are inducible GAL1 promoters, and wherein the growing step occurs in the presence of at least about 2% galactose.

50. A method of producing a diterpene precursor, comprising the steps of:

growing a culture of cells, wherein at least one cell in said culture is the unicellular organism of claim 19, under conditions wherein said diterpene precursor is produced.

51. The method of claim 50, wherein said growing step occurs in the presence of a polyaromatic resin.

52. The method of claim 51, wherein said polyaromatic resin is in a weight-to-volume ratio of about 5%.

53. The method of claim 50, wherein said cell is a yeast.

54. The method of claim 53, wherein said promoter of the nucleic acid sequence encoding said GGPP synthase and said promoter of the nucleic acid sequence encoding said soluble form of said HMG-CoA reductase are inducible GAL1 promoters, and wherein the growing step occurs in the presence of at least about 2% galactose.

55. A method of producing diterpene precursor, comprising the steps of:

growing a culture of cells, wherein at least one cell in said culture is the unicellular organism of claim 24, under conditions wherein said diterpene or diterpene precursor is produced.

56. The method of claim 52, wherein said growing step occurs in the presence of a polyaromatic resin.

57. The method of claim 53, wherein said polyaromatic resin is in a weight-to-volume ratio of at least about 5%.

58. The method of claim 52, wherein said removal comprises filtration or extraction.

59. A method of producing a diterpene or diterpene precursor, comprising the steps of:

growing a culture of cells, wherein at least one cell in said culture is the unicellular organism of claim 32, under conditions wherein said diterpene or diterpene precursor is produced.

60. A method of producing a diterpene, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 18, wherein a metabolic pathway in said cell comprises a process which converts farnesyl pyrophosphate to a triterpene or sterol, and wherein said process is modified, under conditions wherein said diterpene is produced; and

removing said diterpene from said culture.

61. The method of claim 60, wherein said modification of said metabolic pathway occurs at at least one enzyme selected from the group consisting of squalene synthase, squalene epoxidase and lanosterol synthase.

62. The method of claim 60, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme.

63. A method of producing a diterpene precursor, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 21, wherein a metabolic pathway in said cell comprises a process which converts farnesyl pyrophosphate to a triterpene or sterol, and wherein said process is modified, under conditions wherein said diterpene precursor is produced.

64. The method of claim 60, wherein said modification of said metabolic pathway occurs at an enzyme selected from the group consisting of squalene synthase, squalene epoxidase, lanosterol synthase, or a combination thereof.

65. The method of claim 60, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme.

66. A method of producing a diterpene or diterpene precursor, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 32, wherein a metabolic pathway in said cell comprises a process which converts farnesyl pyrophosphate to a triterpene or sterol, and wherein said process is modified, under conditions wherein said diterpene or diterpene precursor is produced.

67. The method of claim 66, wherein said modification of said metabolic pathway occurs at an enzyme selected from the group consisting of squalene synthase, squalene epoxidase, lanosterol synthase, or a combination thereof.

68. The method of claim 66, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme

69. A method of producing a diterpene, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 18, and wherein said cell further comprises a modification of a prenyltransferase, under conditions wherein said diterpene is produced.

70. The method of claim 69, wherein said prenyltransferase is protein farnesyltransferase, protein geranylgeranyltransferase I alpha subunit, protein geranylgeranyltransferase I

beta subunit, protein geranylgeranyltransferase II alpha subunit, or protein geranylgeranyltransferase II beta subunit.

71. The method of claim 69, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme.

72. A method of producing a diterpene precursor, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 21, and wherein said cell further comprises a modification of a prenyltransferase, under conditions wherein said diterpene precursor is produced.

73. The method of claim 72, wherein said prenyltransferase is protein farnesyltransferase, protein geranylgeranyltransferase I alpha subunit, protein geranylgeranyltransferase I beta subunit, protein geranylgeranyltransferase II alpha subunit, or protein geranylgeranyltransferase II beta subunit.

74. The method of claim 72, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme.

75. A method of producing a diterpene, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 18, and wherein a hexaprenylpyrophosphate synthetase is modified, under conditions wherein said diterpene is produced.

76. The method of claim 75, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme.

77. A method of producing a diterpene precursor, comprising the steps of:

growing a culture of cells, wherein said culture comprises at least one cell of claim 21, and wherein hexaprenylpyrophosphate synthetase is modified, under conditions wherein said diterpene precursor is produced.

78. The method of claim 77, wherein said modification comprises an alteration in a nucleic acid sequence which encodes said enzyme, an alteration in expression of a nucleic acid sequence which encodes said enzyme, or an alteration in translation or proteolysis of said enzyme.

79. A method of isolating a diterpene synthase, comprising the steps of:

growing a plurality of cells of claim 21 in the presence of a polyaromatic resin to make a cell/resin mixture, wherein at least one of said cells further comprises at least one isolated and purified nucleic acid sequence of a yeast expression library, wherein the expression of said nucleic acid sequence is regulated by an inducible promoter, under conditions wherein said expression is induced;

filtering said cell/resin mixture;

extracting said cell/resin mixture with alcohol to produce an organic eluent;

analyzing said organic eluent by a screening method, wherein said screening method comprises chromatography, spectroscopy, or a combination thereof, and wherein said screening method identifies said nucleic acid sequence as encoding said diterpene synthase.

20251010 09:07:00